Imbibitions, energy test and accelerated ageing in primed and non-primed seeds of *Peltophorum dubium*

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Abstract: *Peltophorum dubium* seeds were set to imbibe with four treatments, soaked with solution Captan 0.2% under 10 and 27 °C, PEG 6000 -1.0 MPa under 10 and 27 °C. For each treatment there were four replicates with 40 seeds incubated in 9-cm Petri dishes with double filter paper moistened with testing solution. The imbibition curves showed that the final weight increase were from 70% to 150% in the treatments when imbibition entered a lag phase. Seeds were tested for effects on germination of five treatments: control group (non-primed), primed with PEG6000 -1.0 MPa at 10 and 27 °C, primed with Captan 0.2% at 10 and 27 °C. For each treatment, there were three sub-treatments: seeds were soaked in distilled water for 12, 24 and 36h before the energy test. Germination percentages of nonprimed seeds and primed in PEG 27 °C soaked in distilled water during 12 h were the highest, reaching 100%. The lowest germination percentage occurred primed seeds with PEG6000 27 °C and soaked in distilled water during 36 h, which was only 52%. Germination mean time of primed seeds in PEG at 10 °C, soaked 24 h was 1.08 days, mean time of primed seeds in PEG at 27 °C soaked 12 h was 2.42 days. Accelerated ageing results showed low or no germination after ageing 72 h. Control group had a higher germination percentage and seeds were more resistant to deterioration than those in primed groups, both in Petri dish (27 °C) and vermiculate (room temperature).

Keywords: *Peltophorum dubium*; Seeds; Imbibition; Priming; Germination; Ageing
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Introduction

Peltophorum dubium (Spreng) Taub is a perennial wood species, native to Brazilian semi-deciduous forests, about 15–25 m in height and 50–70 cm in diameter, with high quality wood products. This species is listed in endangered species. It is a fast growing and sturdy species that dose well in sunny climate and often used for reforestation of degraded areas. It is also recommended for street planting, for it provides excellent shade (Lorenzi 1992).

The uptake of water by seeds is an essential initial step toward germination. Under optimal supply conditions, the water uptake by seeds is triphasic. Phase I, or imbibition, is largely a consequence of matric forces, and water uptake occurs regardless of whether the seed is dormant or undormant, viable or nonviable. This phase is marked by three characteristics: 1) a sharp front separating wet and dry portions of the seed, 2) continued swelling as water reaches new regions, and 3) an increase in water content of the wetted area. Phase II is the lag phase of water uptake and Phase III is concurrent with radicle elongation and only germination seeds enter (Bewley & Black, 1986).

Seeds treated with osmotic when introduced to water, these seeds, which are nearly all at the same germination stage, show rapid, almost synchronous radicle emergence. Advantages of this treatment--called osmotic priming--are that treated seeds take less time to germinate (Bewley & Black 1986). The use of PEG6000 priming for limited imbibition appears to hold the most potential for inducing early and synchronous germination of seeds. Primed seed with salt solution was effective in increasing seedling emergence and for reducing the time of emergence in summer greenhouse studies (Yoon *et al.* 1997).

Ageing in all organisms is the sum total of the deterioration processes that eventually lead to death. For most plant species, the so-called orthodox species with respect to the response of their seeds to storage conditions, the time taken for half of the population of seeds to become incapable of germinating is much shorter at a higher moisture content and/or temperature.

The ageing of seeds is indicated by delayed germination, slower growth and increased susceptibility to environmental stress, eventually leading to loss of viability (Byrd and Delouche 1971; McDonald & Nelson 1986). Accelerated ageing of seeds at elevated relative humidity and high temperature lost germination and seed viability and vigor. Some reports (Deraman *et al.* 1987; Tarquis and Bradford, 1992; Smok *et al.* 1993; Corbineau *et al.* 1994) have shown that primed seeds are more sensitive to accelerated ageing than nonprimed ones while other finds (Dearman *et al.* 1986) have demonstrated a protective effect of priming on subsequent tolerance to ageing.

The aim of this work is to show imbibition curves of *P. dubium* seeds under different temperatures and solutions as well as germination ability and resistance to ageing.

Materials and methods

Biological material

The seeds of *P. dubium* were provided by Copersucar, São Paulo state, Brazil. These seeds are orthodox and stored in hermetic condition at 5°C throughout the experiments. All the seeds for experiment were previously scarified with 98% sulfuric acid for 15 min to overcome mechanical dormancy (Perez *et al* 1999)

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and washed in tap and distilled water, dried under room temperature.

Basic experimental procedure

The Petri dishes of 9 cm or 15 cm in diameter, with double filter paper in each, were sterilized 2 h at 150°C to minimize the effect of contamination on the seeds during the tests. Vermiculite was also sterilized for the test. PEG6000 solutions were prepared according to the osmotic potential described in Villela *et al.* (1991). Testing seeds were primed in Captan 0.2% solution for 4 h at 27 °C in an aluminum box with double filter paper moistened with Captan 0.2% solution, initial weight was noted down before priming. After priming the seeds were washed and dried, keeping in refrigerator at 27 °C until the final weight returned to the initial weight, and then the seeds were ready for the test. Priming time in Captan solution at 10 °C was 10 h, 24 h in PEG6000 both at10 °C and 27 °C, 24 h in KNO_3.

Imbibition

There were four treatments for the imbibition: seed tested with Captan 0.2% at 10 °C and 27 °C, PEG at 10 °C and 27 °C ,with four replicates for each treatment. Ten seeds were incubated in each replicate 9-cm Petri dish with double filter paper soaked with the testing solution of 4–6 ml. Initial dry weight of the seeds was recorded for each replicate. Final weight was recorded to compare the initial weight every 2 h in the first 24 h during the imbibition, every 6 h in the next 48 h and then every 12 h until the radicle emergence ending the imbibition test. Standard errors should be controlled at 5% among replicates within treatments. For the treatment of Captan 27 °C, it is necessary to record the final weight every hour from 12 h to 24 h during the imbibition test because of the fast water uptaking. New filter paper would substitute the old paper when its color turned yellow. Fungus was removed during the experiment.

Energy test

All the seeds were set for the experiment in Petri dishes of 9 cm diameter, five replicates with 50 seeds after soaking in distilled water during 12, 24 and 36 h.

Germination was observed every 24 h. Germinated seeds with radicle protrusion ≥ 2 mm and with position geotropic curve were eliminated, as well as the dead seeds or those without any condition of germination. The tests were considered to be finished when all seeds had germinated or when they no longer had the possibility to germinate. Final data included total germination (G), mean time (t), germination speed (r) (Labouriau, 1983). Primed seeds and nonprimed seeds were also planted in the experimental garden for field performance. For each group five replicates were made with 100 seeds. Daily observation of seedling emergence ≥ 2 cm was noted. Four weeks later, seedlings were cut for analysis. Final germination percentage and above ground dry matter were collected.

Accelerated ageing

Five groups were chosen for accelerating ageing test: control group, primed in Captan at 10 °C and 27 °C, primed in PEG at 10 °C and 27 °C. For each group, 600 seeds were accelerated ageing in the ageing camera with 100% humidity at 43–45 °C for 24, 48 and 72 h. Seeds were taken out of the camera and fungus was removed before setting to germinate in the incubator (100 seeds) at 27 °C and in the vermiculite (100 seeds) in the laboratory. Daily observation of the germination was necessary and fungus in the seed lots need to be removed.

Statistical analysis

Data are tested using ANOVA (Sokal and Rohlf, 1980).

Results and discussion

Imbibition

Imbibition tests of *P. dubium* seeds in different solutions under different temperatures showed the difference on the process of water uptaking.

Results show that the sharp increase of the weight of seeds with solution Captan at 10 °C in the first 24 h, while the sharp curve increase happened on the seeds with Captan 0.2% at 27 °C in the first 12 h. And imbibition curves of seeds with PEG -1.0 MPa at 10 and 27 °C were sharp in the first 24 h. Then each curve became to fluctuate around its stable line. Radicle emission of seeds with Captan 27 °C was occurred after 24 h of the imbibition.

This imbibition experiment of *P. dubium* finished when the radicle emerged, so the curves show the first and second phases of water uptaking. The imbibition of seeds is an essential and initial step toward germination.

From Fig. 1, the final weight was about 250% of the initial weight of Captan 10 °C, 240% of the initial weight of Captan 27°C, 180% of the initial weight of PEG 10°C, and 170% of the initial weight of PEG 27°C. Then all of the imbibition entered a lag phase and the curves became stable after the sharp increase of the seeds weight. Imbibition in Captan 27°C was much faster than those in PEG solutions due to the water stress on the seeds.

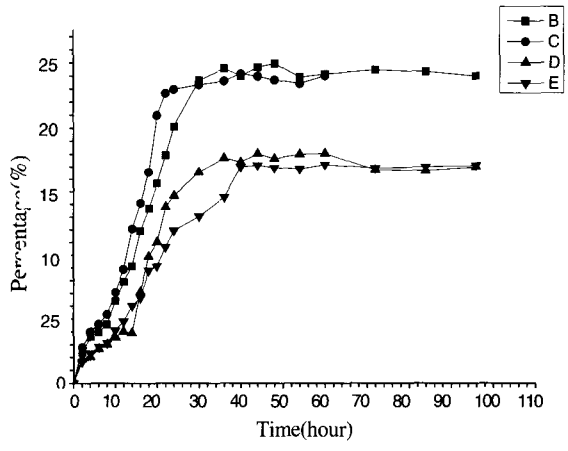

Fig.1 Imbibition curves of *Peltophorum dubium* in different solutions under different temperatures.

B: Captan 10℃, C: Captan 27℃, D: PEG 10℃, E: PEG27℃.

Germination

The germination percentage and speed of the seeds in different treatments and soaked in distilled water were shown in Table 1. Seeds soaked in water for 12 h in control group had the highest germination (100%) and germination speed (0.95 day^{-1}). Seeds primed in PEG 10 °C and 27 °C and soaked in water 12 h also have full germination result (100%). For seeds in control group soaked in water for 24 h, each group had different germination speed. Seeds primed in PEG 27°C soaked in water 12 h had the

slowest germination speed (0.45 day^{-1}) and seeds primed in PEG 10 ℃ soaked during 36 h had the quickest germination speed (0.975 day^{-1}). There was no significant difference in germination percentage between the primed and nonprimed seeds except those that were primed in PEG 27 ℃ and soaked in water during 24 and 36 h and in Captan 10 ℃ and soaked during 36 h. Germination percentage of seeds primed in PEG 27 ℃ soaked in water during 36 h had the lowest value and showed significant difference from other groups. Seeds from control group and primed in Captan 27 ℃ soaked 36 h did not show much difference from other groups except seeds primed in PEG 27 ℃ soaked 36 h. There was no significant difference in germination percentage between treatments of seeds primed in PEG 27 ℃ soaked 24 and 36 h, and in Captan 10 ℃ soaked 36 h.

Table 1. Mean germination percentage and speed for *P. dubium* seeds of nonprimed (control) or primed in Captan (10 or 27 ℃), PEG (-1.0 Mpa) (10 or 27 ℃) soaked in water during 12, 24, 36 h.

Treatment	Germination Percentage (%)*		Germination Speed(days^{-1})	
Control (12 h)	100	A	0.95	A
Primed in Captan 10(12 h)	90	A	0.675	BC
Primed in Captan 27(12 h)	92.5	A	0.575	BC
Primed in PEG 10(12 h)	100	A	0.725	BC
Primed in PEG 27(12 h)	100	A	0.45	C
Control (24 h)	100	A	0.775	AB
Primed in Captan 10(24 h)	90	A	0.675	BC
Primed in Captan 27(24 h)	97.5	A	0.825	AB
Primed in PEG 10(24 h)	97.5	A	0.95	A
Primed in PEG 27(24 h)	65	BC	0.55	BC
Control(36 h)	82.5	AB	0.775	A
Primed in Captan 10(36 h)	75	BC	0.525	C
Primed in Captan 27(36 h)	80	AB	0.575	BC
Primed in PEG 10(36 h)	87.5	A	0.975	A
Primed in PEG 27(36 h)	55	C	0.825	AB
	dms=20.79		dms=0.21	
	F=11.13		F=16.357	
	Fc=2.16		Fc=2.16	

* means followed by the same letter on the same column data differ at $P<0.05$.

Table 1 also gives information about germination speed from seeds in all the groups. There was no significant difference in seed germination speed between control groups, groups primed in Captan (27 ℃) and PEG (10 ℃) which were soaked 24 h, PEG (10 ℃) and 27 ℃ soaked 36 h, but these groups had difference from the other groups. The slowest germination speed, 0.45 day^{-1}, occurred on seeds primed in PEG (27 ℃) soaked 12 h, but its germination percentage was also 100.

Seeds soaked 36 h from control group had lowest germination percentage For seeds primed in Captan (10 ℃), higher germination percentage and speed occurred from seeds soaked during 12 and 24 h. For seeds primed in Captan (27 ℃), 24-h soaking seeds had a higher germination percentage and germination speed. For seeds primed in PEG (10 ℃), 36-h soaking seeds had a lower germination percentage. For seeds primed in PEG (27 ℃), 12-h soaking seeds had a higher germination percentage but lower germination speed.

Seeds soaked 12 h showed no significant difference in germination percentage, but much difference in germination speed, and control group had the highest germination speed and showed significant difference from others.

For seeds soaked 24 h, there were no significant difference in germination percentage between the experimental groups, except PEG (27 ℃) which had the lowest germination percentage, but they had significant difference in germination speed, highest value for treatment of PEG (10 ℃) and the lowest value for treatment of PEG (27 ℃).

For 36-h soaked seeds, the seeds with different treatments of showed significant difference in both germination percentage and germination speeds. Seeds treated with PEG (10 ℃) had the highest germination percentage and germination speed, seeds treated with PEG (27 ℃) had the lowest germination percentage, and seeds treated with Captan (10 ℃) had the lowest value of germination speed.

Field performance

Field performance of primed seeds and nonprimed seeds was observed on germination percentage and above-ground dry matter of 4-week-old seedlings. All the experimental groups showed no significant difference in both germination percentage and dry matter (Table 2). Nonprimed seeds had the highest germination percentage (96.25%) and individual dry matter (0.061 g). Seeds primed in Captan (27 ℃) had the lowest germination percentage (87.5%) and PEG (10 ℃) had the lowest value of dry matter (0.051g).

Table 2. Germination percentage and above ground dry matter of *P. dubium* seeds of different treatments in field performance

Treatment	Germination Percentage (%)		Individual dry matter(g)	
Control	96.25	A	0.061	A
Primed in Captan(10°C)	92.5	A	0.055	A
Primed in Captan(27°C)	87.5	A	0.054	A
Primed in PEG (10°C)	88.75	A	0.051	A
Primed in PEG 27(°C)	95	A	0.056	A
	Dms=9.57		Dms=0.01	
	F=3.03261		F=2.77	
	Fc=3.8		Fc=3.8	

Accelerated ageing

Accelerated ageing on the germination for nonprimed and primed seeds was conducted in both incubator at 27 ℃ and vermiculite at room temperature. Nonprimed seeds after 24 h ageing showed highest germination percentage at 27 ℃ and at room temperature (Table 3). There was no significant difference in germination percentage between the primed groups ageing 24 h at room temperature except the seeds primed in PEG (10 ℃). No significant difference was observed among the groups at room temperature except control group ageing 24 h. All the seeds after aging 24, 48 and 72 h showed very low germination percentage were (Table 3) and had a trend of decrease in germination percentage with the increase of ageing period. After ageing 72 h, almost no germination occurred at 27°C and at room temperature. Seeds of control group were found more resistant to ageing deterioration during the test, still the germination percentage was only 20% at 27 ℃ after ageing 24 h and as lower as 5% after ageing 48 h. Seeds of control group had only 6% germination at room temperature after ageing 24 h..

Table 3. Germination percentage of primed and nonprimed *P. dubium* seeds in incubator and vermiculite after accelerated ageing.

Ageing Time (h)	Treatments	Germination percentage (%)			
		Petri dish (27 ℃)		Vermiculite (room temperature)	
	Control	20	A	6	A
	Primed in Captan(10°C)	5	B	3	AB
24	Primed inCaptan(27°C)	4	B	2	AB
	Primed in PEG(10°C)	3	B	3	AB
	Primed in PEG(27°C)	4	B	1	B
	Control	5	B	2	AB
	Primed in Captan(10°C)	2	B	1	B
48	Primed inCaptan(27°C)	1	B	1	B
	Primed in PEG(10°C)	2	B	0	B
	Primed in PEG(27°C)	4	B	1	B
	Control	1	B	0	B
	Primed in Captan(10°C)	1	B	0	B
72	Primed in Captan(27°C)	0	B	0	B
	Primed in PEG(10°C)	0	B	0	B
	Primed in PEG(27°C)	1	B	0	B
		Dms=5.91		Dms=4.48	
		F=17.4801		F=3.75918	
		Fc=2.12		Fc=2.12	

However, primed *P. dubium* seeds are more sensitive to accelerate ageing than the nom-primed seed. The same phenomenon was observed with seeds of carrot (Dearman *et al.* 1987 and sunflower (Smok *et al.* 1993) and leek (Corbineau *et al.* 1994).

Conclusions

The imbibition process of *P. dubium* was affected by both solution concentrations and temperatures. The lowest temperature and osmotic potential decreased the imbibition rate.

Germination in the incubator at 27 ℃ and field performance showed the nonprimed seeds of *P. dubium* had a higher germination percentage and above ground individual dry matter compared with those physiologically primed in solutions Captan 0.2% and osmoconditioned in solutions PEG6000 -1.0 MPa. It is difficult to conclude that osmotic priming with PEG 6000 or priming with Captan 0.2% had a better effect on the germination of *P. dubium* seeds, at least at the early stage, priming did not show evidence to improve the seedling's growth. Probably more similar experiments need to be conducted on this area to compare those attempts on some vegetable species and tree species.

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